REMARKS/ARGUMENTS

Claims 1-11 are currently pending in the above-identified application. Claims 1, 2, and 9 have been amended as set forth in detail below. Support for these amendments is identified in the following remarks. No new matter is added by these amendments.

Rejections under 35 U.S.C. § 112, First Paragraph

Enablement

Claims 1-11 stand rejected under 35 U.S.C. §112, first paragraph, the Examiner believing that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. Applicants respectfully traverse the instant rejection.

It is well-settled that the Examiner bears the initial burden of providing evidence or reasoning why a pending claim does not meet the requirements of 35 U.S.C. § 112, first paragraph. In particular, "a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented *must* be taken as in compliance with the first paragraph of § 112 *unless* there is a reason doubt the objective truth of the statements contained therein that must be relied upon for enabling support." *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971) (emphasis original). It is incumbent upon the Examiner to explain why the truth or accuracy of these statements should be doubted and to provide acceptable evidence or reasoning in support. *See id.*

In the present case, in attempting to support the rejection, the Examiner makes the following allegations:

(A) "the specification does not teach as to how lack of $p27^{kipl}$ in one cell type only would result in the increase in the proliferation of thymocytes ...";

(B) "the specification does not teach as to how an artisan would have administered an antibody to an animal such that the gene encoding $p27^{kip1}$ in the thymus gland would be altered ...";

(C) "the art of gene delivery in vivo to a particular cell type and also in general is highly unpredictable ...";

(D) "the method of ex vivo therapy is also unpredictable"; and

(E) "the making of transgenic non-human animals is unpredictable as recognized in the prior art at the time of the invention ...."

The following addresses each of the points noted above. However, before discussing the Examiner's rejections, Applicants emphasize that the present invention is based in part on the surprising discovery that thymocyte proliferation can be increased by inhibiting the function of the $p27^{kip1}$ gene. Because $p27^{kip1}$ is phylogenetically conserved, both structurally and functionally, it is submitted that this is a fundamental phenomenon that can be used to increase thymocyte proliferation regardless of the particular animal species involved, and would be reasonably recognized as such by the skilled artisan.

Although the invention in based on a fundamental discovery in the biology of cell cycle regulation, its practical applicability must be exemplified in particular experimental systems. For practical reasons, the initial experimental proof is generated using a well-established animal model. As such, Applicants demonstrated the ability of the methods of the present invention to increase thymocyte proliferation using a mouse transgenic model. As demonstrated by the studies described in the specification, disruption of $p27^{kip1}$ function caused an increase in thymocyte number via an increase in cell proliferation. It is respectfully submitted that the teachings provided in the specification, in combination with the general knowledge in the art at the time of the invention, are sufficient to allow one of skill in the art to use the methods as presently claimed.

A. With regard to the Examiner's first allegation (that the specification does not teach how lack of $p27^{kip1}$ in one cell type only would result in the increase in the proliferation

of thymocytes), Applicants note that embodiments that are inoperative, and would be recognized as such by the skilled artisan with expenditure of no more effort than normally required in the art, are not encompassed by a claim. MPEP § 2164.08(b), citing *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984). Applicants submit that the skilled artisan would readily understand that altering $p27^{kip1}$ function in a cell which is not a thymocyte or in the thymocyte lineage would not increase thymocyte proliferation. Therefore, Applicants believe that such embodiments do not render the claims non-enabled, irrespective of the Examiner's broad interpretation of the claims.

While Applicants respectfully disagree with this basis for the Examiner's rejection, but in order to further expedite prosecution of the instant application, Applicants have amended claim 1 to recite "altering an endogenous gene encoding $p27^{kip1}$ in a ~~somatic~~ thymocyte, or a multipotent cell that differentiates into a thymocyte." Support for this amendment is found in the specification at, *e.g.*, page 16, lines 30-33; and page 18, lines 1-5 and 15-18. For the reasons stated above, Applicants believe do not believe that this amendment narrows the scope of claim 1 or any dependent claim.

B. As to the Examiner's contention that the specification is not enabling because "the specification does not teach as to how an artisan would have administered an antibody to an animal such that the gene encoding $p27^{kip1}$ in the thymus gland would be altered," Applicants respectfully remind the Examiner that interpretation of a claim during patent examination, while generally broad, must still be reasonable (*see* MPEP § 2111). It is respectfully submitted that an interpretation of the phrase in claim 1 -- "altering an endogenous gene encoding $p27^{kip1}$" -- as including administration of an antibody that would alter the gene, to be unreasonable. To support his contention, the Examiner refers to the specification, which describes various means for inhibiting the function of $p27^{kip1}$, including the use of an antibody. (*See* specification at page 16, line 36, bridging to page 17, line 1.) However, this description does not refer to altering of the $p27^{kip1}$ gene itself, but inhibition of $p27^{kip1}$ function by using an antibody directed to the protein. Also, Applicants again note that embodiments which are inoperative, and which are readily determined as such by the skilled artisan, are not encompassed

by a claim. MPEP § 2164.08(b). Thus, the skilled artisan, reading claim 1 in light of the specification, would readily understand that a method of altering the $p27^{kip1}$ gene with an antibody is not encompassed by claim 1.

C. With respect to the Examiner's statements regarding alleged unpredictability of gene delivery *in vivo*, the Examiner cites to Miller and Vile (*FASEB J.*, 9:190-199, 1995) (hereinafter "Miller"). Applicants believe that the Examiner's reliance on Miller is misplaced. Miller discusses targeted vectors for gene delivery in the context of therapeutic efficacy. Miller does not state that currently available delivery systems are ineffective, but simply reviews how such systems "can be manipulated to improve their targeting to specific cell types." Abstract (emphasis added). Further, Applicants note that all questions of enablement are evaluated with respect to the claimed subject matter, *see* MPEP § 2164.08, and the interpretation of the claims must be consistent with the interpretation that those skilled in the art would reach (*id.* at § 2111, citing *In re Cortright*, 165 F.3d 1353, 1359, 49 USPQ2d 1464, 1468 (Fed. Cir. 1999). In the present case, the claims recite "increasing the proliferation of thymocytes." The claims do not recite any particular threshold for targeting specificity or of clinical therapeutic efficacy. Therefore, the skilled artisan would reasonably interpret the claims as requiring some increase in the proliferation of thymocytes, but not necessarily in a statistically significant clinical therapeutic regime. Consequently, Applicants submit that Miller is insufficient to rebut Applicants' presumption of enablement.

D. Applicants also respectfully disagree with the Examiner's statement that, assuming "the $p27^{kip1}$ in a cell is altered first in vitro and the cell is administered, ... the method of ex vivo therapy is also unpredictable." Applicants again note that, for the reasons set forth above, the skilled artisan would reasonably interpret the claims as requiring some increase in thymocyte proliferation, but not necessarily in a therapeutic regime. Further, the "specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public." MPEP § 2164.05(a). It is respectfully submitted that, as of the effective filing date of the application, *ex vivo* methods that

could be used to achieve the recited methods were known, including, *e.g.*, methods for (1) isolating hematopoietic progenitor cells from an animal, including cells that differentiate into thymocytes, (2) transfection of the cells with a vector for alteration of a particular gene, and (3) re-administration of the cells to the animal. Applicants note that the Examiner has not provided any evidence to the contrary. Further, whether to use autologous, xenogeneic, or allogeneic cells is based on factors well-known to the skilled artisan, and claims "are not rejected as broader than the enabling disclosure under 35 U.S.C. 112 for noninclusion of limitations dealing with factors presumed to be within the level of ordinary skill in the art." MPEP § 2164.08.

E. Finally, Applicants respectfully disagree with the Examiner's contention that the making of transgenic non-human animals is unpredictable.

The Examiner cites alleged limitations in the art of making transgenics of species other than mouse, including "[l]onger gestation times, reduced litter sizes, number of fertilized eggs required for micro injection and relatively low efficiency of gene integration and method of introduction of transgenes." However, while such factors may suggest that the routine methods for making non-mouse transgenics are time-consuming and may not be optimally efficient, they do not, *a priori*, mean that a transgenic animal of a species other than mouse cannot be predictably achieved.

Applicants also believe the Examiner's reliance on Cameron (*Molecular Biotechnology* 7:253-265, 1997) to be misplaced. Cameron is directed to expression of a heterologous transgene, and the Examiner cites to Cameron as stating, "Well regulated transgene expression is the key to successful transgenic work ...." However, because the methods of the present invention are directed to <u>disruption</u> of the $p27^{kip1}$ gene, not expression, well-regulated expression of the transgene is irrelevant.

The Examiner also believes the claims are nonenabled because introduction of foreign DNA into a fertilized oocyte "may result in random integration of the exogenous DNA into host chromosomal DNA ...." However, methods for the structural disruption of a p27 gene require a directed integration event, not random integration. Therefore, the skilled artisan is able

to predict, for example, the number of microinjections that might be required to identify those animals in which an integration event structurally disrupting a p27 gene would have occurred.

The Examiner further contends that the art of culturing and maintaining ES cells in culture, and the art of transgenesis based on ES cells, was also unpredictable at the time of the invention. Applicants respectfully note that ES and ES-like cells had been identified and isolated in a variety of animal species prior to the filing date of the earliest claimed priority of the present application. See, *e.g.*, Thomson *et al.*, *Proc. Natl. Acad. Sci. USA* 92:7844-48, 1995 (rhesus monkey); Doetschman *et al.*, *Dev. Biol.* 127:224-227, 1988 (hamster, four ESC lines); Iannaccone *et al.*, *Dev. Biol.* 163:288-292, 1994 (rat); Graves and Moreadith, *Mol. Reprod. Dev.* 36:424-433, 1993 (rabbit); Wheeler, *Reprod. Fertil . Dev.* 6:563-68 (1994) (pig); Wakamatsu and Ozato, *Mol. Mar. Bio. Biotechnol.* 3:185-91, 1994 (fish: medaka); Sukoyan *et al.*, *Mol. Reprod. Dev.* 33:418-31, 1992 (mink, ten ESC-like lines); Petitte and Yang, U.S. Patent No. 5,340,740, 1994 (avian), *see also* Wheeler, WO 94/26884, 1994, at, *e.g.*, pp. 14, lines 21-28 and 28, lines 11-18. These ES lines have been demonstrated to share characteristics in common with mouse ES cell lines that have been used to construct transgenic mice. These characteristics include, for example, pluripotency, development of tissues of different types when allowed to differentiate in culture, and chimera formation when injected into a developing blastocyst. *See, e.g.*, Iannaccone *et al.*, *Dev. Biol.* 163:288-292, 1994 (rat); Schoonjans *et al.*, *Mol. Reprod. Dev.* 45:439-43, 1996 (rabbit); Wheeler, *Reprod. Fertil. Dev.* 6: 563-68, 1994 (pigs); Hong *et al.*, *Proc. Nat. Acad. Sci. USA* 95: 3679-3684, 1998 (fish: medaka). Given the demonstrated success of isolating and identifying ES cells from non-human animals other than mouse, it is submitted that those skilled in the art would recognize that germ line transmission of a transgene could predictably be achieved using such ES cells from species other than mouse.

Furthermore, Applicants note that, at the time of filing the parent of the present application, the skilled artisan would have recognized that the production of transgenic animals with a structurally disrupted $p27^{kip1}$ gene could be achieved by methods other than implanting ES or ES-like cells or microinjecting DNA into embryos. One method, in particular, known prior to the filing of the parent of the instant application was the transfer of genetically modified nuclei to

enucleated oocytes. At the time of filing, nuclear transfer was proven to produce viable mammalian offspring, *see, e.g.*, Campbell, *et al.*, *Nature* 380:64-66, 1996. In addition, methods for genetically modifying nuclei via homologous recombination were similarly well established, *see, e.g.*, Doetschman *et al.*, *supra*; Thomas and Capecchi, 1987, *supra*; Thomas and Capecchi, *Nature (London)* 346:847-50, 1990. Consequently, the skilled artisan would recognize that the genetically modified nuclei from an animal cell line, including gene knockouts, could be transferred to enucleated oocytes, thus avoiding chimeric generation and ensuring germ line transmission. Moreover, the more recent disclosure of targeted gene deletion in transgenic sheep using nuclear transfer, Denning *et al.*, *Nature Biotechnol.* 19:559-62, 2001, demonstrates that a method for producing gene knockouts in animals other than mice known by the skilled artisan at the time of filing, does in fact generate viable animals with the gene deletions.

For the reasons set forth above, Applicants believe claims 1-11 to be enabled by the specification as filed, and that the Examiner has not met the requisite burden to establish a reasonable basis to question the enablement provided for the claimed invention. Therefore, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 1-11 and nonenabled under 35 U.S.C. § 112, first paragraph.

Written Description

Claims 1-11 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner states that the invention encompasses "transgenic knockout animals whose phenotypes and characteristics may not be known ..." and that "there is unpredictability of phenotypic effects caused by variation in the genetic background used to generate or propagate gene targeted models ...." The Examiner further bases the rejection on the contention that the effects of inactivating a gene, such as the $p27^{kip1}$ gene in the transgenic non-human animals encompassed by the invention, can not be predicted. Applicants respectfully traverse this rejection.

The written description inquiry must be analyzed with respect to the claimed subject matter. *See Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991) (stating that the "invention is, for purposes of the 'written description' inquiry, whatever is now claimed") (emphasis original). In the present case, independent claim 1 recites a "method for increasing the proliferation of thymocytes in a non-human animal comprising altering an endogenous gene encoding $p27^{kipl}$ in a thymocyte, or a multipotent cell that differentiates into a thymocyte, of the animal to cause a functional deficiency of cyclin-dependent kinase inhibitor function of $p27^{kipl}$, thereby increasing the proliferation of thymocytes in the animal" (emphasis added). It is noted that claim 1 does not recite any other effect of altering $p27^{kipl}$ other than increasing the proliferation of thymocytes. Thus, any other phenotypic effects of $p27^{kipl}$ inhibition are irrelevant to the question of written description.

Further, Applicants note that, in view of the specification as filed, the skilled artisan would reasonably believe that inhibiting $p27^{kipl}$ function in a thymocyte or thymocyte precursor cell would predictably increase thymocyte proliferation. $p27^{kipl}$ belongs to a family of proteins (Kips) that negatively regulate G1 cyclin-dependent kinases by binding preferentially to cyclin/CDK complexes. (*See* specification at page 2, lines 11-22.) It was also known as of the filing date of the instant application that Kips are highly conserved phylogenetically. Accordingly, in light of the Applicants' disclosure, which demonstrates that disruption of the $p27^{kipl}$ gene causes an increase in thymocyte proliferation, the skilled artisan would believe that Applicants were in possession of a method of increasing the proliferation of thymocytes by altering an endogenous $p27^{kipl}$ gene in a thymocyte or thymocyte precursor cell to cause a functional deficiency of the CDK inhibitor function of $p27^{kipl}$.

For the reasons set forth above, Applicants believe claims 1-11 satisfy the written description requirement under 35 U.S.C. § 112, first paragraph. Therefore, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 1-11 as lacking written description under 35 U.S.C. § 112, first paragraph.

Rejections under 35 U.S.C. §112, Second Paragraph

Claims 1-11 are rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 4

The Examiner contends that claim 4 is indefinite because it recites the phrase "the gene encoding $p27^{kip1}$ is altered by insertion of a positively selectable marker." The Examiner states that "a marker is usually a protein and it is unclear as to how a protein would be inserted in the gene encoding p27kip1." Applicants respectfully traverse this rejection.

It is well-established that the determination of whether a claim is definite depends on whether those skilled in the art would understand the scope of the claim when the claim is read in light of the specification. *See North Am. Vaccine, Inc. v. American Cyanamid Co.*, 28 USPQ2d 1333, 1339 (Fed. Cir. 1993). Thus, the interpretation of a claim during patent examination must be consistent with the interpretation that those skilled in the art would reach (MPEP § 2111, citing *In re Cortright*, 165 F.3d 1353, 1359, 49 USPQ2d 1464, 1468 (Fed. Cir. 1999), "taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by ... applicant's specification" (MPEP § 2111, citing *In re Morris* , 127 F.3d 1048, 1054-55, 44 USPQ2d 1023, 1027-28 (Fed. Cir. 1997)). Further, it is noted that while claims are typically interpreted broadly during examination, such interpretation must still be reasonable. *See* MPEP § 2111, citing *In re Hyatt* 211 F.3d 13671372, 54 USPQ2d 1664, 1667 (Fed. Cir. 2000).

Applicants respectfully disagree with the Examiner's interpretation of "marker" in claim 1 as including a protein. First, Applicants note that claim 4 recites the term "selectable marker" (emphasis added). The phrase "selectable marker" is a term of art, well-known as of the effective filing date of the instant application, that refers to a gene whose expression allows one to identify cells that have been transformed or transfected and containing the marker gene. The

specification states, for example, that "$p27^{kipl}$ may be deleted and replaced by at least one selectable marker gene." (Specification at page 4, lines 11 and 12.) Further, the term "selectable markers" is used in *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.* eds., Cold Spring Harbor, N.Y., 1989), and are described therein. Sambrook *et al.* state, for example, that [t]o identify ... transformants, selectable markers encoded by the plasmid are used. ... The most commonly used selectable markers are genes that confer resistance to antibiotics such as ampicillin, tetracycline, chloramphenicol, and kanamycin (neomycin)." *Id.* at 1.5 (emphasis added). Consistent with this plain meaning in the art, the specification states, for example, that "$p27^{kipl}$ may be deleted and replaced by at least one selectable marker gene." (Specification at page 4, lines 11 and 12.) Moreover, irrespective of the well-known meaning for "selectable marker," but simply given the context of the term "marker" in the claim and the written description provided in the specification, the skilled artisan would not reasonably interpret a "marker" for insertion into a gene as including a protein.

In view of the above, Applicants submit that the Examiner's interpretation of the term "marker" in claim 4 is neither reasonable nor consistent with the interpretation that the skilled artisan would reach. Therefore, Applicants believe claim 4 is definite and respectfully request the Examiner to reconsider and withdraw the rejection of claim 4 as indefinite under 35 U.S.C. § 112, second paragraph.

Claim 1

The Examiner, stating that claim 1 recites "the gene encoding $p27^{kipl}$ in a somatic cell," believes claim 1 to be indefinite because a somatic cell, as broadly interpreted, could contain a gene encoding $p27^{kipl}$ in a plasmid in addition to the endogenous gene of the somatic cell.

Applicants respectfully direct the Examiner's attention to page 54 of the application as filed, where claim 1 clearly recites "altering an endogenous gene encoding $p27^{kipl}$ in a somatic cell" (emphasis added). Because claim 1 recites "endogenous gene encoding $p27^{kipl}$," and is therefore definite, Applicants traverse the instant rejection. Applicants

respectfully request the Examiner to reconsider and withdraw the rejection of claim 1 as indefinite under 35 U.S.C. § 112, second paragraph.

Claim 9

The Examiner believes claim 9 to be indefinite because it recites the term "a gene encoding $p27^{kipl}$ in the cell." As with claim 1, the Examiner states that somatic cell, as broadly interpreted, could contain a gene encoding $p27^{kipl}$ in a plasmid in addition to the endogenous gene of the somatic cell. Applicants respectfully traverse the instant rejection.

Applicants note that in the present case claim 9 incorporates all of the limitations of claim 1, which recites "altering an endogenous gene encoding $p27^{kipl}$." Applicants also note that the additional limitations in claim 9 refer to characteristics of the plasmid, not the somatic cell, *i.e.*, the distance between the negatively selectable marker and the altered gene encoding $p27^{kipl}$, whereby the distance "is sufficient to allow homologous recombination between the altered gene encoding $p27^{kipl}$ and a gene encoding $p27^{kipl}$ in the cell." It is submitted that the distance (between the marker and the altered gene encoding $p27^{kipl}$) that would allow homologous recombination between the altered gene and a gene encoding $p27^{kipl}$ does not depend on whether the gene encoding $p27^{kipl}$ is endogenous or contained in a plasmid. Thus, Applicants believe that the skilled artisan, reading claim 9 in light of the specification, would understand the scope of claim 9.

While Applicants do not agree with the Examiner's rejection, but in order to further expedite prosecution of the instant application, Applicants have amended claim 9 to recite "the endogenous gene encoding $p27^{kipl}$ in the cell." For the reasons set forth above, Applicants believe that this amendment does not narrow the scope of claim 9.

In view of the above remarks and amendment, Applicants believe claim 9 to be definite. Therefore, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claim 9 as indefinite under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. §102

Claims 1-11 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Roberts *et al.* (U.S. Patent No. 5,958,769, dated August 28, 1999, filing date January 18, 1996).

Applicants assert that the instant invention is not anticipated by Roberts *et al.* A Declaration Under 37 C.F.R § 1.131, establishing that the present invention was either reduced to practice prior to the effective date of Roberts *et al.* , or that the present invention was conceived of prior to the effective date of Roberts *et al.* coupled with due diligence from prior to the effective date to a subsequent reduction to practice or to the filing of the present application, will be submitted shortly with a Supplemental Amendment.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance upon submission of the Declaration Under 37 C.F.R § 1.131, establishing a date of invention prior to the filing date of Roberts *et al.* If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: 6 May 2004

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